View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Cell, Vol. 84, 659–662, March 8, 1996, Copyright ©1996 by Cell Press

Clustering Membrane Proteins: It's All Coming Together with the PSD-95/SAP90 Protein Family

Minireview

Stephen N. Gomperts
Neuroscience Program
University of California, San Francisco
San Francisco, California 94143–0450

Neurons differentially localize and cluster membrane proteins. Spatial localization and clustering of ion channels and neurotransmitter receptors are necessary for neurons to fire and propagate action potentials and for normal synaptic transmission to occur. For example, voltage-dependent sodium and potassium channels must aggregate at the nodes of Ranvier of myelinated axons for action potentials to propagate. To accommodate the diversity of each neuron's synaptic inputs, including glutamate-mediated excitatory and GABA-mediated inhibitory inputs, a postsynaptic neuron expresses appropriate neurotransmitter receptors at each synaptic junction. Neurons often achieve additional complexity by expressing different receptors sensitive to the same neurotransmitter at a given synapse. The spatially localized aggregation of receptors ensures a rapid and robust response to released neurotransmitter. In this regard, changes in receptor cluster density will strongly impact postsynaptic responses. How receptors and ion channels cluster and localize in the CNS is an interesting question. Recent evidence suggests that a number of homologous synaptic proteins including PSD-95/SAP90 may play a role in this process. PSD-95/SAP90 interacts with both NMDA receptors (Kornau et al., 1995; Niethammer et al., 1996) and Shaker-type potassium channels (Kim et al., 1995) and has the potential to cluster many membrane-associated proteins in neurons throughout the brain. Supporting a role for PSD-95/SAP90 in localizing signal transduction machinery as well, Brenman et al. (1996) show in this issue of *Cell* that PSD-95/SAP90 also interacts with neuronal type nitric oxide synthase. The PDZ domains of PSD-95/SAP90 mediate these interactions, functioning as novel protein-binding modules. This minireview will summarize these recent results in the context of what is known about receptor and ion channel clustering in the nervous system.

Receptor Clustering at the Neuromuscular Junction

Our current knowledge of receptor and ion channel localization and clustering derives largely from studies of the neuromuscular junction (NMJ) (reviewed by Froehner, 1993). At the NMJ, nicotinic acetylcholine receptors (nAChRs) are localized at the motor endplate opposite the presynaptic nerve terminal. These receptors are tightly clustered, achieving a density of approximately 10,000 receptors/μm^2. This is in stark contrast to the thousand-fold lower receptor density found just micrometers away, beyond the motor endplate. Recent work has shown that the NMJ-specific intracellular protein rapsyn participates both in clustering and in localizing nAChRs (reviewed by Apel and Merlie, 1995). Cotransfection experiments in fibroblasts have demonstrated that rapsyn can cluster nAChRs and that rapsyn can independently cluster dystroglycan, a member of a complex of transmembrane and extracellular proteins known as the dystrophin–glycoprotein complex (DGC) (Apel et al., 1995). The DGC is found along the sarcolemma of skeletal muscle and associates with both the cytoskeleton and the extracellular matrix. By binding rapsyn and synapse-specific proteins, such as utrophin and β2-syntrophin, the DGC may localize the nAChR clusters to the synapse.

The DGC also appears to localize signal transduction machinery. Neuronal-type nitric oxide synthase (nNOS) associates with the DGC and is concentrated at the sarcolemma of fast twitch skeletal muscle fibers (Brenman et al., 1995). Many of the muscular dystrophies, which cause progressive muscle weakness and premature death, derive from genetic errors in members of the DGC (reviewed by Campbell, 1995). Duchenne muscular dystrophy, which targets dystrophin, disrupts both the DGC and the localization of nNOS at the sarcolemma (Brenman et al., 1995). nNOS may well be important in skeletal muscle function. In the mature animal, nitric oxide (NO) appears to promote skeletal muscle relaxation, and during development is implicated in both myoblast fusion and activity-dependent NMJ synaptic suppression (references can be found in Brenman et al., 1996). The latter finding suggests that despite its sarcolemmal distribution, nNOS may function in NMJ synaptic signaling.

Receptor and Ion Channel Clustering in the CNS

In the central nervous system, proteins with rapsyn-like function are currently being identified and characterized. One such protein is gephyrin, a 93 kDa membrane-associated intracellular protein that appears to be essential for the clustering of glycine receptors at inhibitory synapses (reviewed by Kuhse et al., 1995). Until recently, gephyrin was the only putative rapsyn-like protein identified in the central nervous system.

Glutamate-releasing excitatory synapses are abundant in the brain, but a molecular basis for glutamate receptor clustering has been lacking. Fast glutamatergic transmission is mediated by two pharmacologically distinct receptors known as the NMDA and the AMPA/kainate receptors. The NMDA receptor has been the subject of considerable interest because of its roles in synaptic plasticity (reviewed by Nicoll and Malenka, 1995) and excitotoxicity (reviewed by Choi, 1988). To examine NMDA receptor links to cellular proteins, Kornau et al. (1995) recently set out to isolate proteins interacting with the cytoplasmic carboxy-terminal tails of two of its possible subunits, NR1 and NR2. Their protein interaction screens, involving both yeast two-hybrid and biochemical methods, identified a previously characterized synaptic protein known both as PSD-95 and as SAP90. PSD-95/SAP90 and NMDA receptors colocalize at putative synapses in hippocampal pyramidal cells (Kornau et al., 1995), supporting an in vivo association of these proteins.

Using similar techniques, Kim et al. (1995) recently showed that PSD-95/SAP90 and two related family members, hdlg/SAP97 and clone 5, also interact with

Figure 1. Domain Map of PSD-95/SAP90

All members of the PSD-95/SAP90 protein family share this domain sequence. PDZ, SH3, and yeast guanylate kinase homology (GuK) domains are indicated.

several Shaker-type potassium channel subunits. Consistent with an in vivo association, PSD-95/SAP90 and Shaker-type potassium channel subunit Kv1.2 colocalize to basket cell nerve terminals in the cerebellum.

PSD-95/SAP90 Family Members

PSD-95 (*P*ost-*S*ynaptic *D*ensity protein 95 kDa) was first identified as an abundant cytoskeleton-associated protein found in the postsynaptic density fraction of rat synaptosomes (Cho et al., 1992). It was independently identified as SAP90 (*S*ynapse *A*ssociated *P*rotein 90 kDa) (Kistner et al., 1993). Its developmental expression appears to parallel CNS synaptogenesis. Despite its name, PSD-95/SAP90 has been found in both postsynaptic and presynaptic junctions.

PSD-95/SAP90 is one of a family of membrane-associated proteins. Family members share three PDZ domains in their amino terminus, an SH3 domain, and a carboxy-terminal yeast guanylate kinase (GuK) homology domain (Figure 1). PDZ (also known as GLGF or DHR) domains are 90 amino acid repeats of previously unknown function. While they are found in a number of unrelated proteins, PDZ domains are named after three of the homologous proteins that contain them: *P*SD-95/SAP90, *D*lg (discs large), and *Z*O-1 (zonula occludentes-1). PSD-95/SAP90 family SH3 domains and GuK domains implicate these proteins in cell signaling, but this has not been demonstrated. SH3 domains are frequent sites of protein–protein interactions. Although the function of the GuK homology domain is unclear, it may well be important since many lethal mutations of *dlg* occur in this region. Yeast guanylate kinase produces GDP and ADP from GMP and ATP, but there has been to date no demonstration that the yeast GuK homology domains of PSD-95/SAP90 family members are catalytically active. PSD-95/SAP90 and most family members studied can bind GMP with micromolar affinity, but they do not appear to bind ATP tightly (Kistner et al., 1995).

Most known PSD-95/SAP90 family members localize at sites of cell–cell contact. The protein product of the Drosophila *dlg* gene is found at septate junctions in many endothelial tissues (Woods and Bryant, 1991) and at a subset of glutamatergic neuromuscular junctions (Lahey et al., 1994). Septate junctions are thought to be the invertebrate equivalent of vertebrate tight junctions and function in cell-cell adhesion. *dlg* mutations have a larval phenotype characterized by cell overproliferation, loss of cell polarity, extensive loss of cell contact in imaginal discs, and a defective postsynaptic apparatus at that subset of NMJs at which Dlg is expressed. ZO-1 is found at vertebrate tight junctions (Willott et al., 1993), which are thought to play a role both in tissue compartmentalization and in maintaining the apical-basolateral polarity of epithelial cells. Other family members have also been identified (e.g., Brenman et al., 1996).

Heterotypic Interactions with PSD-95/SAP90

PSD-95/SAP90 interacts with both NMDA receptors and Shaker-type potassium channels. The first two PDZ domains of PSD-95/SAP90 can bind NMDA receptor subunits (Niethammer et al., 1996). Kornau et al. (1995) showed that PSD-95/SAP90's second PDZ domain binds the carboxy-terminal seven amino acids of specific NMDA receptor subunits. NMDA receptor subunits that interact with PSD-95/SAP90 share a similar seven amino acid terminal sequence; subunits missing this sequence did not associate with PSD-95/SAP90. Kim et al. (1995) demonstrated that Shaker-type potassium channel subunits can also bind both the first and the second PDZ domains of PSD-95/SAP90 family members. They additionally demonstrated that the carboxy-terminal four amino acids of the channel subunits are necessary and the last 11 amino acids sufficient for binding to occur. Those NMDA receptor subunits that bind and the Shaker-type potassium channel subunits share a carboxy-terminal threonine/serine X valine (T/SXV) motif. Kim et al. (1995) showed that site-directed mutagenesis of these residues (T653A; V655A; V655E) abolished binding of Shaker-type subunit Kv1.4 to PSD-95/SAP90. Interestingly, a large number of membrane-associated proteins, including many neuronal ion channels and synaptic receptors, share this terminal T/SXV motif (Kornau et al., 1995). Candidate partners of PSD-95/SAP90 family members and other PDZ-containing proteins include three sodium channel α subunits and a number of G-protein coupled receptors, including the β1 adrenergic receptor and serotonin receptors 2A and 2C. Whether these putative partners do in fact bind PDZ-containing proteins remains to be demonstrated.

Homotypic Interactions with PSD-95/SAP90

Like PSD-95/SAP90 family members, neuronal-type nitric oxide synthase (nNOS) has a PDZ domain. In addition to its distribution at the sarcolemma of fast-twitch skeletal muscle (see above), it is found in many neuronal cell types throughout the peripheral and central nervous systems (reviewed by Garthwaite and Boulton, 1995). In the CNS, nNOS appears to modulate synaptic transmission. Activation of NMDA receptors and other calcium-permeable channels has been shown to stimulate nNOS (reviewed by Garthwaite and Boulton, 1995). Although nNOS has a PDZ domain, it does not appear to directly interact with the NMDA receptor (Kornau et al., 1995). However, Brenman et al. (1996) demonstrate that, like NMDA receptors, nNOS binds PSD-95/SAP90, and interacts as well with novel family member PSD-93, apparently via PDZ–PDZ domain interactions. Supporting an in vivo association, these authors show that nNOS and PSD-95/SAP90 colocalize in neurons of embryonic rat and immunoprecipitate as a complex from adult rat cerebellum. nNOS binding is mediated by one of the PDZ domains of PSD-95/SAP90 that bind NMDA receptor subunits. The fact that nNOS has no terminal T/SXV motif demonstrates that binding is not restricted to this consensus motif. Interestingly, PSD-95/SAP90 only binds nNOS isoforms that contain a PDZ domain, suggesting a PDZ-PDZ homotypic interaction. Although NMDA receptor terminal T/SXV motifs and nNOS compete in vitro for binding at PSD-95/SAP90's second PDZ domain, NMDA receptor subunits can independently

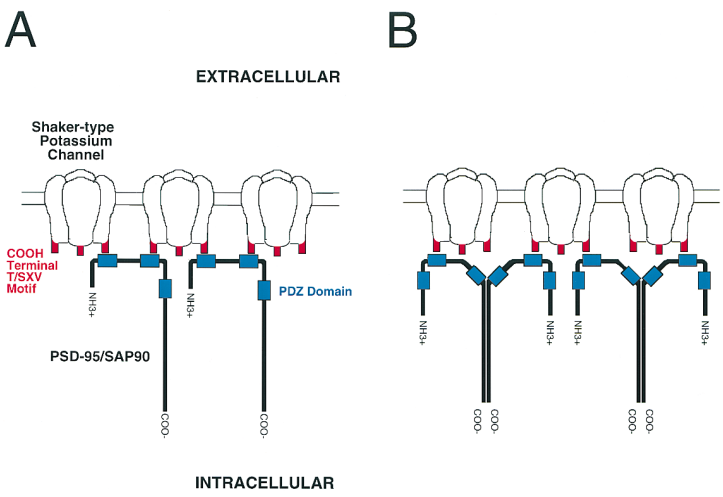

Figure 2. Two Possible Models for the Clustering of Shaker-Type Potassium Channels and PSD-95/SAP90

The tetrameric structure of the potassium channel permits interaction with multiple PSD-95/SAP90 proteins.

(A) Because each Shaker-type subunit can bind PSD-95/SAP90 at either of its first two PDZ domains, one PSD-95/SAP90 molecule tethers two channels together.

(B) PSD-95/SAP90 multimers aggregate with Shaker-type subunits.

Note that these models are not mutually exclusive.

bind PSD-95/SAP90's first PDZ domain (Niethammer et al., 1996). It is therefore unlikely that binding one molecule precludes the binding of the other. The interaction of PSD-95/SAP90 with both NMDA receptors and nNOS suggests that PSD-95/SAP90, perhaps as an oligomeric complex (see below), may bind and associate these receptors with their signal transduction machinery. PSD-95/SAP90 may thus provide a scaffold for spatial localization of molecular cascades.

Neither PSD-95/SAP90 nor PSD-93 is found in muscle, yet nNOS associates with the dystrophin-glycoprotein complex (DGC). Brenman et al. (1996) show that skeletal muscle α1-syntrophin, a cytoplasmic component of the DGC, mediates this interaction. As with PSD-95/SAP90, it appears that PDZ-PDZ domain interactions underlie the protein association.

Functional Significance of PSD-95/SAP90 Binding Shaker-Type Subunits

PSD-95/SAP90's biochemical binding and colocalization with the NMDA receptor and Shaker-type subunits provide correlative evidence of a direct association in vivo. More importantly, Kim et al. (1995) showed that PSD-95/SAP90 and the Shaker-type subunits functionally interact. Shaker-type channel subunits spontaneously form tetrameric, functional potassium channels in heterologous cells (Stuhmer et al., 1989). Transfection of one channel subunit alone into COS7 cells led to a diffuse membrane distribution, while transfection of PSD-95/SAP90 alone resulted in a diffuse cytoplasmic distribution. In contrast, cotransfection of the channel subunit and PSD-95/SAP90 resulted in the formation of membrane-associated, micron sized clusters comprised of both proteins. This finding shows that PSD-95/SAP90 may function to cluster Shaker-type potassium channels, and vice versa.

How do the clusters form? One model (Figure 2A) for clustering builds on the observation that both PDZ1 and PDZ2 domains of PSD-95/SAP90 can bind Shaker-type subunits (Kim et al., 1995). One PSD-95/SAP90 protein can therefore bind two different channels, and because each channel is comprised of four subunits, each channel can bind up to four PSD-95 molecules, resulting in the observed aggregation. Another model (Figure 2B) suggests that clustering occurs because PSD-95/SAP90 intrinsically self-aggregates into a multimer that aggregates with other membrane proteins (Kim et al., 1995). Since the former model requires the presence of both PDZ1 and PDZ2 domains in one PSD-95/SAP90 molecule, it predicts that a PSD-95/SAP90 protein missing either domain will not cluster Shaker subunits when cotransfected into COS7 cells.

What's Next?

A number of questions remain. Because PSD-95/SAP90 can bind both NMDA receptors and nNOS, it may associate these receptors with their signal transduction machinery. Does PSD-95/SAP90 associate NMDA receptors with nNOS in vivo? The evidence for colocalization in neurons remains controversial (reviewed by Garthwaite and Boulton, 1995). If so, what other molecular partners do PSD-95/SAP90 and its family members colocalize, and are such associations subject to regulation? The implication that PSD-95/SAP90 associates nNOS with Shaker-type potassium channels is curious, since there are no experimental data to support this. While this may be the case in vivo, it is also possible that family members other than PSD-95/SAP90 are the biologically relevant ligands for nNOS and the Shaker-type and NMDA receptor subunits. The possibility that different PSD-95/SAP90 family members have distinct binding partners could also permit different family members to cluster these membrane proteins in different subcellular locations. However, if PSD-95/SAP90 family members recognize a large variety of membrane proteins, they are unlikely, on their own, to provide the specificity needed for subcellular localization.

Because heterologous clustering of receptors and ion channels may provide signaling diversity, it would be interesting to know whether PSD-95/SAP90 family members can bind and colocalize two distinct membrane proteins. For example, a G protein–coupled receptor may cluster with a channel whose properties are modulated by G proteins. It would also be interesting to determine whether PSD-95/SAP90 binding modulates the function of its binding partners.

How general is the observation that PSD-95/SAP90 functions in clustering? Although PSD-95/SAP90 can

cluster Shaker-type potassium channel subunits in vitro, whether NMDA receptor subunits and nNOS are similarly influenced by PSD-95/SAP90 has yet to be demonstrated. It is noteworthy that PSD-95/SAP90 and Shaker-type subunits only cluster when expressed together. This coclustering may play a role in the assembly of the synapse. While PSD-95/SAP90 appears to function in aggregating ion channels and perhaps other membrane-associated proteins, how are clusters, once formed, anchored in place? It is conceivable that PSD-95/SAP90, like the NMJ protein rapsyn, interacts with proteins that provide both cytoskeletal and extracellular matrix interactions.

While we are still at an early stage in understanding the mechanisms that underlie the clustering and localization of membrane-associated proteins in neurons, the discovery of the PSD-95/SAP90 protein family opens up an exciting new direction of research. The key role of PSD-95/SAP90's PDZ domains in mediating its interactions with NMDA receptors, Shaker-type potassium channel subunits, and nNOS identifies the PDZ domain as a protein binding module. Its presence in non-neuronal proteins, such as Dlg and ZO1, may help clarify their functions.

Acknowledgments

I thank Roger Nicoll for his valuable input and sponsorship.

Selected Reading

Apel, E.D., and Merlie, J.P. (1995). Curr. Opin. Neurobiol. *5*, 62–67.

Apel, E.D., Roberds, S.L., Campbell, K.P., and Merlie, J.P. (1995). Neuron *15*, 115–126.

Brenman, J.E., Chao, D.S., Gee, S.H., McGee, A.W., Craven, S.E., Santillano, D.R., Wu, Z., Huang, F., Xia, H., Peters, M.F., Froehner, S.C., and Bredt, D.S. (1996). Cell *84*, this issue.

Brenman, J.E., Chao, D.S., Xia, H., Aldape, K., and Bredt, D.S. (1995). Cell *82*, 743–752.

Campbell, K.P. (1995). Cell *80*, 675–679.

Cho, K-O., Hunt, C.A., and Kennedy, M.B. (1992). Neuron *9*, 929–942.

Choi, D.W. (1988). Neuron *1*, 623–634.

Froehner, S.C. (1993). Annu. Rev. Neurosci. *16*, 347–368.

Garthwaite, J., and Boulton, C.L. (1995). Annu. Rev. Physiol. *57*, 683–706.

Kim, E., Niethammer, M., Rothschild, A., Jan, Y.N., and Sheng, M. (1995). Nature *378*, 85–88.

Kistner, U., Garner, C.C., and Linial, M. (1995). FEBS Lett. *359*, 159–163.

Kistner, U., Wenzel, B.M., Veh, R.W., Cases-Langhoff, C., Garner, A.M., Appeltauer, U., Voss, B., Gundelfinger, E.D., and Garner, C.C. (1993). J. Biol. Chem. *268*, 4580–4583.

Kornau, H.-C., Schenker, L.T., Kennedy, M.B., and Seeburg, P.H. (1995). Science *269*, 1737–1740.

Kuhse, J., Betz, H., and Kirsch, J. (1995). Curr. Opin. Neurobiol. *5*, 318–323.

Lahey, T., Gorczyca, M., Jia, X.-X., and Budnik, V. (1994). Neuron *13*, 823–835.

Nicoll, R.A., and Malenka, R.C. (1995). Nature *377*, 115–118.

Niethammer, M., Kim, E., and Sheng, M. (1996). J. Neurosci., in press.

Stuhmer, W., et al. (1989). EMBO J. *8*, 3235–3244.

Willott, E., Balda, M.S., Fanning, A.S., Jameson, B., Van Itallie, C., and Anderson, J.M. (1993). Proc. Natl. Acad. Sci. USA *90*, 7834–7838.

Woods, D.F., and Bryant, P.J. (1991). Cell *66*, 451–464.